is appreciated that such relative affinities may be used to select molecules capable of binding to the FR and to select conjugates that include FR receptor binding moieties.

[0009] In another embodiment, compounds, compositions, and methods are described herein that include a drug delivery conjugate of the formula

$$AL(D)_m$$

are described wherein A is a folate receptor binding antifolate; L is a monovalent or multivalent linker, comprising at least one releasable linker, each D is a drug; and m is an integer from 1 to about 3. It is to be understood that when m is greater than 1, each drug D is independently selected in each instance. In other words, D may be the same drug or be different drugs in each instance.

[0010] In another embodiment, the compositions and methods described herein are useful for treating one or more pathogenic populations of cells in a patient. The compositions include a therapeutically effective amount of one or more conjugates described herein, optionally in combination with one or more carriers, excipients, and/or diluents, or any combinations thereof. The methods include the step of administering an effective amount of one or more conjugates described herein and/or one or more compositions described herein. In another embodiment, the compositions and methods are useful for treating cancer, and other diseases.

[0011] In another embodiment, uses of the compounds and compositions in the manufacture of medicaments are described herein, where the medicaments include a therapeutically effective amount of one or more conjugates described herein and/or one or more compositions described herein for treating one or more pathogenic populations of cells in a patient. It is to be understood that the medicaments may be used in any of the methods described herein for treating one or more pathogenic populations of cells in a patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. **1**. Panel A, KB cell-based relative affinity assay. Panel B, cell-free relative affinity assay (isolated plate-bound folate receptor). Both assays were performed in the absence of serum while the plates were sitting on a bed of ice. FA, (●). EC145, (■).

[0013] FIG. **2**. Panel A. Pte acid on ice. Panel B, Pte at 37° C. Panel C, LV on ice. Panel D, LV at 37° C. Each assay was conducted using adherent KB cells as the FR source without serum in the test medium. FA=Pte-γGlu, (●); LV, (○); Pte (■).

[0014] FIG. **3**. Panel A, EC72 on ice. Panel B, EC72 at 37° C. Panel C. EC17 on ice. Panel D, EC17 at 37° C. Each assay was conducted using adherent KB cells as the FR source without serum in the test medium. FA, or Pte-γGlu, (●); EC72, (○); EC17 (■).

[0015] FIG. **4**. Panel A, binding of FA to KB cells in the presence of increasing amounts of fetal bovine serum (FBS). Panels B and C, RA of FA and EC145, respectively (percent serum: 0% (●), 10% (■), 25% (▲), 50% (□), 75% (▽), 100% (○)). Panel D effect of 0% or 10% scrum on the RA of EC140.

[0016] FIG. **5**. Activity of compounds against subcutaneous KB tumors (2 μmol/kg TIW/2 weeks: vertical dotted line indicates last day of dosing): (a) Controls; (b) EC0282 (CB3717) 0/5 complete responses; (c) EC145 2/5 complete responses; (d) EC0284 5/5 complete responses.

[0017] FIG. **6**. Percent change in weight of treated animals in each test group, (2 μmol/kg TIW/2 weeks; vertical dotted line indicates last day of dosing): (a) Controls; (b) EC0282 (CB3717) 0/5 complete responses; (c) EC145 2/5 complete responses; (d) EC0284 5/5 complete responses.

[0018] FIG. **7**. Relative affinity assay (10% serum/FDRPMI): FA (●) 1.000; EC284 (■) 0.148; EC283 (○) 0.002.

[0019] FIG. **8**. Relative affinity assay (10% serum/FDRPMI): (a) FA (●) 1.000; (b) EC282 (●) 0.148 FIG. **9** Activity of EC282 against KB cells (72 h continuous Assay): Panel A. IC_{50} 5 nM. Panel B. IC_{50} 19 nM.

[0020] FIG. **10**. Activity of EC0284 against KB cells (2 h treatment/72 h Assay): (a) EC0284+excess folic acid; (b) EC0284.

DETAILED DESCRIPTION

[0021] In one embodiment, drug delivery conjugates of the formula

$$AL(D)_m$$

are described wherein A is a folate receptor binding antifolate: L is a monovalent or multivalent linker, comprising at least one releasable linker; each D is a drug; and m is an integer from 1 to about 3. It is to be understood that when m is greater than 1, each D may be the same drug or be a different drug from another in each instance. In another embodiment, the monovalent or multivalent linker L includes at least two releasable linkers. In one variation of each of the foregoing, the monovalent or multivalent linker includes at least one releasable linker that is not a disulfide.

[0022] In another embodiment, the conjugates described herein include an antifolate having a relative affinity for the folate receptor, as compared to folic acid, of at least about 0.1, at least about 0.2, at least about 0.25, or at least about 0.5, at one or more of the temperatures described herein. In another embodiment, the conjugates described herein have a relative affinity for the folate receptor, as compared to folic acid, of at least about 0.05, at least about 0.1, at least about 0.2, at least about 0.25, or at least about 0.5, at one or more of the temperatures described herein. In another embodiment, the compounds, compositions, and methods that include conjugates comprising a folate receptor binding antifolate, at least one releasable linker, and one or more drugs, where the antifolate has a high relative affinity for the folate receptor, as compared to folic acid, at temperatures above 4° C., such as at temperatures above 20° C., at temperatures above 25° C., at temperatures above 30° C., and/or at temperatures that are physiologically relevant, such as physiological temperatures in mammals. Also described herein are compounds, compositions, and methods that include conjugates comprising a folate receptor binding antifolate, at least one releasable linker, and one or more drugs, where the conjugate has a high relative affinity for the folate receptor, as compared to folic acid, at temperatures above 4° C., such as at temperatures above 20° C., at temperatures above 25° C., at temperatures above 30° C., and/or at temperatures that are physiologically relevant, such as physiological temperatures in mammals. In general, the conjugates described herein are covalent conjugates; however, it is to be understood that the drugs forming part of the conjugates described herein may include other bond forms, including but not limited to complexes, such as metal chelates, and the like.